


REMARKS

The information contained in the computer readable form of Application No. 08/924,777 was prepared through the use of the software program "PatentIn" and was identical to that of the paper copy. This amendment contains no new matter.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

  
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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**In the Specification:**

Paragraph beginning at line 16 of page 56 has been amended as follows:

To facilitate efficient secretion of human interleukin-1 $\beta$  (hIL-1 $\beta$ ), a DNA fragment encoding mature hIL-L $\beta$ , amino acids 117-269 of the unprocessed protein was linked to sequences encoding the prepro leader peptide (amino acids 1-31) of human parathyroid hormone (hPTH). To remove the potential for N-linked glycosylation, amino acids 7 through 9 of mature hIL-1 $\beta$  were changed from the glycosylation consensus sequence, Asn-Cys-Ser, to Gln-Ala-Ser. The preproIL-1 $\beta$  coding region was PCR amplified and inserted into the Nco I and BamH I restriction sites of the MFG retroviral vector according to the methods of Dranoff, *Proc. Natl. Acad. Sci. U.S.A.* 90: 3539-3543 (1993) and Robbins et al., *Annals of the New York Academy of Sciences* 716: 72-89 (1993). The upstream primer (gccaccATGgTACCTGCA; SEQ ID NO:7) contained nucleotides 1-12 of the 5' end of the hPTH leader sequence (shown in caps.), with the fourth residue changed from an A to a G and an additional 6 nucleotides to accommodate the recognition sequence for Neo I (underlined). The downstream primer (AGCACAGGATCCTCTGGGTAC; SEQ ID NO:8) corresponded to sequences in the pCDNA1 vector adjacent to the 3' end of the hIL-1 $\beta$  coding region which were modified to contain a BamH I recognition sequence (underlined). To allow positive selection of retrovirally transduced cells, a DNA fragment containing an internal ribosome entry site, as described by Ghattas et al., *Mol Cell. Biol.* 11: 5848-5849 (1991). (IRES) 5' to the cDNA encoding neomycin phosphotransferase (*neo*<sup>r</sup>) was inserted into the BamH I site of the MFG-hIL-1 $\beta$  plasmid, immediately downstream of the hIL-1 $\beta$  coding region. The resulting plasmid construct (pDFG-hIL-1 $\beta$ -neo) allows for expression of both the hIL-1 $\beta$  and *neo*<sup>r</sup> gene products from a single polycistronic transcript initiated from the upstream retroviral long terminal repeat as shown by Robbins et al., 715: 72-89 (1993), Tahara et al., *J. Immunol.* 154: 6466-6477 (1994) and Zitvogel et al., *Hum Gene Ther.* 5:1493-1506 (1994).